APHIS 81-27
September 1976

The Surface Sterilization Of Pink Bollworm Eggs And Spread Of A Cytoplasmic Polyhedrosis Virus In Rearing Containers

Agricultural Research Service
United States Department of Agriculture

In Cooperation with
University of Arizona Agricultural Experiment Station

Printed by: Animal and Plant Health Inspection Service
U.S. Department of Agriculture

CONTENTS

Note: The use of trade names in this article is for purposes of scientific accuracy and is not intended as endorsement of products.

THE SURFACE STERILIZATION OF PINK BOLLWORM EGGS AND SPREAD OF A CYTOPLASMIC POLYHEDROSIS VIRUS IN REARING CONTAINERS

By Fred D. Stewart, *research entomologist*, Marion R. Bell, *research entomologist*, and A. J. Martinez, *biological research technician, Western Cotton Research Laboratory, Western Region, Agricultural Research Service, U.S. Department of Agriculture, Phoenix, Ariz.*, Jon L. Roberson, *entomologist* and Anna M. Lowe, *insect production worker, APHIS, PPQP, PP Pink Bollworm Rearing Facility, Western Region, Agricultural Research Service, U.S. Department of Agriculture, Phoenix, Ariz.*

INTRODUCTION

The mission of the USDA, APHIS Pink Bollworm Rearing Facility in Phoenix, Ariz., is to provide sterile moths for release as a possible control technique for the pink bollworm, *Pectinophora gossypiella*, in the San Joaquin Valley of California. The production of laboratory-reared research insects has been hampered in the past by the debilitating effects of a cytoplasmic polyhedrosis virus (CPV) that has persisted in the facility for several years.

New moth cultures known to be free of CPV were introduced only to be diseased again within a short time. The APHIS facility has unsuccessfully employed modifications of a formaldehyde treatment (Vail et al., 1968; Bullock et al., 1969) and a sodium hypochlorite treatment (Ignoffo, 1966) to surface disinfect pink bollworm eggs.

Ignoffo and Adams (1966) reported on a CPV isolated from the pink bollworm larvae reared under laboratory conditions and determined that the virus was cross-infective for the cabbage looper. The CPV in the APHIS culture is similar to or identical to the one described by Hemple and Adams and is also cross-infective for the cabbage looper.

The most severe effect of the CPV on pink bollworms is the mortality of larvae and pupae. Other observed effects include reduction in pupal weight, retardation of pupation, and adult emergence (Ignoffo and Adams, 1966). Bullock et al. (1970) showed that CPV infection decreased the weight of larvae, adult fecundity and longevity. Bell (unpublished data) found that the percent protein and lipid content of infected pupae was decreased and, furthermore, the pupal weight was decreased by CPV infection but was not affected by increasing viral concentration in the diet. The highly infectious nature of the virus was indicated by Bell (unpublished data) who demonstrated that a 900-fold difference existed between the effective concentration (EC_{50}) and the lethal concentration (LC_{50}).

Several researchers have considered the problem of generation-to-generation transmission of CPVs infecting the larvae of different species of Lepidoptera. Aruga and Nagishima (1962) suggested that the CPV of the silkworm, *Bombyx mori*, was transmitted to the next generation within the egg; Hukuhara (1962) concluded that the CPV of the silkworm was transmitted in an occult form in the germ plasm. Adams et al. (1969) identified a CPV virion within pink bollworm eggs. Bullock et al. (1969) stated that CPV on the surface of the pink bollworm egg was an important means of transmitting the virus. Sikorowsky et al. (1973) concluded that the major mode of generation-to-generation transmission of the CPV of *Heliothis virescens* was on the surface of the egg. Mery and Dulmage (1975) stated that the transmission of CPV between generations of *H. virescens* was not transovarial but the result of contamination of the eggs during oviposition.

Tests were conducted to determine at what stages in a pink bollworm mass rearing procedure the viral contamination was occurring, the extent of spread of the disease in rearing containers, how generation-to-generation contamination was occurring, and to attempt to apply the results to improving procedures in the rearing facility.

Research was supported in part by Cotton Incorporated, Raleigh, N.C.

MATERIALS AND METHODS

Pink Bollworm eggs were obtained from the APHIS Methods Development Bollworm Rearing Laboratory located in Phoenix. The APHIS facility treated 1 to 3 million eggs simultaneously on circular oviposition pads for 10 minutes in 1 percent sodium hypochlorite solution maintained at 30°C. The detached eggs were separated from the hypochlorite solution by pouring the solution into a stainless steel sieve that retained the eggs. The treated eggs were then rinsed in water and held in water or suspended in 0.1 percent sterile agar solution for later use. The number of eggs was estimated by multiplying the volume of eggs by 18,000 eggs/ml. The treated eggs were dispensed into rearing containers with 10.0 ml pipettes.

Pink bollworm and cabbage looper, *Trichoplusia ni*, eggs that were known to be free of CPV were obtained from the Western Cotton Research Laboratory (WCRL) on oviposition pads that had been surface disinfected by submersion in a 10 percent formaldehyde solution for 45 minutes and then rinsed for 45 minutes in running tap water. Numbers of treated WCRL eggs and untreated APHIS eggs (not removed from the egg pads) were estimated visually or by weight. These eggs were infested into 473cc of diet in 1892cc rearing containers by stapling the pads to the inside of the rearing container lids.

Larval diets used to rear pink bollworms were obtained from APHIS and WCRL. Both diets were essentially the same as described by Ouye (1962) and, unless stated otherwise, were formulated with formaldehyde for its antimicrobial action. The APHIS diet contained Calco Oil Red N-1700® dye which is used as a marker to distinguish released moths from wild moths in the field (Graham and Mangum, 1971). The APHIS diet was dried for 72 hours before being finely shredded and placed in rearing containers. The WCRL diet was dried for 24 hours, coarsely shredded, dried an additional 24 hours and then transferred to rearing containers. Wheat germ diet (Ouye, 1962), formulated with ascorbic acid and without formaldehyde, was used in tests when cabbage looper larvae were reared. All rearing and tests were conducted at 30°C and ambient relative humidity.

A phase-contrast microscope was used for the detection of CPV in adult fecal material and smears of midguts from pink bollworm and cabbage looper larvae. The observation of polyhedral inclusion bodies (PIBs) with a hexagonal appearance was the criterion used for determining infection in larvae and adults. Also, it was often possible to identify infected cabbage looper larvae by the gross symptomology which included retarded growth and a creamy-white midgut that gave the larvae a pale appearance.

TESTS AND RESULTS

Sources of Contamination

The larval diet and the eggs used to infest rearing containers during the APHIS pink bollworm rearing procedure were suspected of being two possible sources of CPV contamination. The following test was replicated with eggs and diet from nine different days. The test consisted of infesting 473cc diet in 1892cc containers containing APHIS or WCRL diet with pink bollworm eggs obtained from either APHIS or WCRL. The amount of diet used in each replicate was determined by the number of eggs available for infestation. The eggs were infested at the rate of 10 eggs/g of diet except in tests 7-9 when 4.2 WCRL eggs/g of diet were used. Following a 10-day incubation period, midguts of 10 larvae from each treatment were removed and examined.

The results (table 1) indicate that both the APHIS diet and eggs were contaminated with CPV during the period the experiments were conducted. The APHIS diet was found to be contaminated on 8 of the 9 days tested

Table 1.—Infection of APHIS pink bollworm larvae exposed to APHIS and WCRL diet

Test number	Container size (cc)	Wt. of diet (g)	Pink bollworm larvae infected with CPV			
			APHIS eggs infested on		WCRL eggs infested on	
			APHIS diet	WCRL diet	APHIS diet	WCRL diet
1	1892	700	+	+	+	–
2	1892	700	+	+	+	–
3	1892	700	+	+	–	–
4	1892	700	+	+	+	–
5	1892	350	–	–	+	–
6	1892	350	–	+	+	–
7	473	150	–	–	[1] +	[1, 2] +
8	473	150	+	–	+	–
9	473	150	+	–	+	–

[1] 4.2 WCRL eggs/g of diet in tests 7-9.
[2] Possible result of aseptic techniques that inadvertently occurred in setting up this particular container.

and the APHIS eggs were contaminated only 5 of the 9 days. However, contamination from one or both sources occurred in every replicate.

Adequacy of Existing Egg Treatment Procedures

Attempts were made to determine if the egg surface disinfection technique employed by APHIS was sufficient to eliminate CPV contamination. Samples of 54,000 to 180,000 pink bollworm eggs treated with hypochlorite were obtained from APHIS. Each sample was rinsed three times in sterile distilled water and homogenized in a 40 ml Ten Broeck homogenizer. A 0.1 ml aliquot of egg homogenate was spread on the surface of 50 (22cc) plastic diet cups which contained 9cc of wheat germ diet with the formaldehyde deleted. One neonatal cabbage looper larva was transferred to each of 50 treated and 50 untreated cups and incubated at 30°C. The presence of CPV was determined by the examination of 20 or more midguts/treatment/replicate after 7 days incubation.

The results (table 2) indicated that the CPV persisted after egg surface treatment and that the contamination was transferred with the eggs at the times the rearing containers were infested. Cabbage looper larvae in the controls were not observed to be infected.

Table 2.–Percent infection of T. ni larvae fed egg-homogenates

Test	No. of pink bollworm eggs	Volume of homogenate	Total larvae examined	Percent infected with CPV
1	180,000	40	21	80.9
2	180,000	30	27	77.8
3	90,000	24	20	80.0
4	54,000	25	20	100.0
Controls	–	25	25	0.0

Modification of the Sodium Hypochlorite Egg Treatment

Based on the results of the adequacy of existing egg treatment procedures, tests were conducted with modified egg treatments to determine if transovarial transmission occurred. A surfactant, agitation (Martignoni and Milstead, 1960), and a formaldehyde treatment (Vail et al., 1968) were added to the basic hypochlorite treatment. In addition, aseptic techniques were employed whenever possible and all floating eggs, scales, etc., were carefully decanted away from submerged eggs and discarded. Specifically, the components of the treatments consisted of submersion in a solution of 1.0 percent hypochlorite plus 0.025 percent Tween 80® for 15 minutes; submersion in a solution of 9.25 percent formaldehyde plus 0.025 percent Tween 80® for 10 minutes; and constant manual agitation with a stirring rod was given to loose eggs at the rate of ca. 30 revolutions per minute. A minimum of 200 ml of solution was used/treatment. Treatments are summarized as follows:

A. Unagitated sodium hypochlorite
B. Unagitated sodium hypochlorite, unagitated formaldehyde
C. Unagitated sodium hypochlorite, agitated formaldehyde
D. Agitated sodium hypochlorite
E. Agitated sodium hypochlorite, unagitated formaldehyde
F. Agitated sodium hypochlorite, agitated formaldehyde

Untreated eggs from APHIS were obtained from moths in oviposition cages that were set up at the APHIS facility on 7 consecutive days. The egg pads from 7 different ages of moths were then separated into 7 groups, each group contained 1 egg pad from each age of moths. One group served as an untreated control to determine if the virus was present prior to egg treatment. Eggs were incubated at 30°C and allowed to hatch. A single neonatal pink bollworm larva was transferred to each of 30 plastic cups (22cc) containing 9 ml of WCRL pink bollworm diet prepared without formaldehyde and incubated at 30°C.

As a part of each treatment, except the control, egg pads were first rinsed for 5 minutes in running tap water (29±1°C) to remove loose debris, including scales, eggs, and fecal material, then placed in 2.0 liters of 1 percent

hypochlorite (29±1°C) and agitated until the eggs were free from the pads. Following each hypochlorite or formaldehyde treatment, each solution was discarded by decanting and the treated eggs were transferred to a 500 ml beaker and washed several times with total volume of 2.0 liters of 29±1°C tap water. During the washes, the eggs were retained in the beaker by an organdy cloth secured with a rubber band.

Following the completion of each treatment, ca. 100-250 eggs from each treatment were transferred to petri dishes containing nutrient agar or APHIS diet (formaldehyde deleted) and the percent hatch of eggs was determined after incubation at 27°C for 7 days. A bioassay of an egg homogenate was conducted as stated above for every treatment in each experiment (replicated 3 times). Each bioassay was conducted with a homogenate of ca. 54,000 eggs brought up to a total volume of 15 ml with sterile distilled water. Approximately 7,200 treated eggs from each treatment were used to infest a single 1892cc container that contained ca. 750g WCRL pink bollworm diet.

In this test no viral infection of pink bollworm larvae was detected and the remaining larvae were allowed to pupate. The pupae were collected from the diet cups and individually incubated in clean 22cc plastic containers. The meconia and feces were examined microscopically for PIBs.

CPV infection was not detected among cabbage looper larvae, nor were PIBs found in the meconia and feces of the adult loopers from the bioassays of any of the modified treatments. PIBs were not found in the midguts of pink bollworm larvae from any of the treatments. PIBs were found in the feces of control pink bollworm adults in only one of the three replicates. The absence of CPV in the other two controls was unexpected, but was probably related to the small number of pink bollworm larvae used and the removal of the neonates from the contaminated egg pads. The hatch rate, 87 to 96 percent, of treated pink bollworm eggs indicated that no appreciable abuse to the eggs occurred during any of the treatments.

The absence of viral disease in all egg-surface treatments, which involved a total of 324,000 eggs, suggested that: (1) the virus contamination was present on a portion of the egg that was subject to control by sodium hypochlorite, (2) the 1.0 percent sodium hypochlorite treatment alone was sufficient to eliminate viral contamination from eggs, and (3) therefore, it would be possible to eliminate the contamination from the large number of eggs in a mass-rearing facility if the differences between the APHIS production treatment and these tests were identified and corrected.

Comparison of APHIS and Modified Egg Treatment Techniques

The egg treatment procedure at the APHIS facility was reexamined to detect where the virus contamination might be occurring. We observed that a thin layer of floating material consisting of eggs, scales, and larvae accumulated on the surface of the hypochlorite solution. Since the results of the previous tests strongly suggested that the CPV contamination persisted on portions of the floating materials not in contact with the hypochlorite solution, an experiment was conducted to compare the efficiency of the APHIS egg treatment with that of the unagitated 1 percent hypochlorite treatment of the previous tests.

An egg pad from each of 7 different ages of moths was cut into 10 equal portions and 1 portion of each pad was placed in each of 10 rearing containers (1892cc) containing 750g of WCRL pink bollworm diet. This was the untreated control (unwashed).

A second sample, consisting of 15 egg pads from the same ages of moths, was subjected to a 1 percent hypochlorite treatment. The eggs and debris were collected in a stainless steel sieve, simulating the egg treatment procedures used by APHIS. Another sample of 15 egg pads was collected and was treated with 1 percent hypochlorite plus 0.025 percent Tween 80® (same as treatment A, preceding section). Eggs collected from these two treatments were then used to infest 10 rearing containers (ca. 7,200 eggs/container). Each rearing container was then placed in an open-top, 11.4-liter container provided with a sheet of 8 mm thick Hexcel® on the bottom as a pupation site. After 20 days, pupae were collected and placed in sealed cages for emergence.

The presence of CPV was determined by microscopic examination of smears from each rearing container and the bioassay of an egg homogenate from each hypochlorite treatment. In addition, the meconia and feces of adults from each pink bollworm emergence container were washed from the surface with 30 ml of sterile distilled water after all moths had emerged. One ml of the wash solution from each container was spread and allowed to dry on the surface of nine 237cc cups containing 30-40g of wheat germ diet (formaldehyde deleted). Twenty-five neonatal cabbage looper larvae were placed in each container and incubated at 30°C for 7 days. In addition to observing the gross symptoms, the midguts of 5 cabbage looper larvae from each treatment were examined for CPV.

The results are summarized in table 3 and show that the APHIS treatment was inadequate in eliminating the viral contamination. However, essentially the same treatment with the addition of a surfactant and the removal of floating debris by decantation eliminated the viral contamination from eggs.

Comparison of the two methods used to detect CPV in table 4 shows that the most reliable method was the examination of looper larvae fed fecal material from pink bollworm moths.

Table 3.–Summary of test comparing APHIS NaClO treatment and the modified treatment

Diagnostic technique for CPV	Egg treatment		
	Untreated	APHIS treated	WCRL treated
Egg homogenates		positive	negative
Microscopic examination of pink bollworm larvae	positive	positive	negative
No. contaminated diet containers/total containers	20%	[1]10%	0.0%
Percent infected of pooled observations[2]	1.5%	1.4%	0.0%
Fecal wash	positive	positive	negative
No. contaminated containers/total containers	[3]100%	[1]10%	[3]0%

[1]The same container was positive in both diagnoses.
[2]Minimum of 100 larvae/treatment, minimum of 10 larvae from each of 10 containers.
[3]Container lost due to fungus.

Table 4.–Observed incidence of infection of 10-day-old pink bollworm larvae by cytoplasmic polyhedrosis virus following establishment of various ratios of treated to untreated 3-day-old larvae in mass-rearing containers

Treatment (3 days)		Incidence of CPV infection at 10 days	Increase in average disease incidence
No. clean larvae	No. virus-fed larvae[1]	Average percent infected[2, 3]	
100	0	0	–
98	2	20 a	10×
96	4	51 ab	13×
92	8	66 ab	8×
84	16	87 b	5×

[1]Virus-fed larvae averaged 86% infected at 10 days.
[2]Mean % infected of 3 replications (1 cup/treatment/replicate).
[3]Means not followed by same letter are significantly different at the 95% level.

Spread of CPV in Rearing Containers

Although the APHIS pink bollworm culture was chronically infected with CPV over a longer period of time, there appeared to be less effect of the disease on larval growth in this culture compared to the effect shown in other studies when larvae were fed cytoplasmic polyhedra (Bell, unpublished data). From these observations it seemed probable that the high incidence of CPV in larval rearing containers might be caused by the spread of the disease between individuals within the rearing containers.

Neonatal larvae were transferred to individual 22cc cups containing either diet (1 ml/cup) with 4×10^3 PIBs/ml or untreated diet. The methods of Bell (unpublished data) were used for the collection and standardization of PIBs. The WCRL diet was formulated without formaldehyde. Preliminary tests indicated that a 3-day feeding period was necessary to obtain 100 percent infection of 10-day-old larvae. Therefore, 3-day-old larvae were used to infest 450 ml mass-rearing cups, each containing 100g of WCRL diet (5 cups/replicate) with various ratios of larvae which were fed on treated diet to those fed on untreated diet (100 total larvae/cup). Care was taken when transferring the larvae from the individual cups not to transfer any diet with the larvae; however, possible external viral combination of these larvae could not be excluded. In order to determine the incidence of infection of the

larvae used to infest the mass-rearing cups, 30 of the 3-day-old larvae from the virus-treated diet were transferred to individual cups containing 6cc of untreated diet and examined for presence of PIBs at 10 days. At 10 days, a sample of 30 larvae from each cup was microscopically examined for presence of PIBs. The test was replicated 3 times.

The results indicated that the incidence of CPV infection of 10-day-old larvae within the mass rearing containers was significantly increased by, and directly related to, the number of virus-fed larvae infested at 3 days (table 4). The greatest increase in average disease incidence of larvae observed in these tests was 51 percent at 10 days for containers that initially held 4 percent CPV treated 3-day-old larvae (a 13-fold increase). The average weights and standard deviations of a single sample (30 larvae) from a treatment indicating 100-percent infection at 10 days, and a sample from the control cup were 20.9 mg ± 7.5 and 25.5 mg ± 5.5, respectively. For comparison, the average weight of a sample of 30 larvae which were fed for 3 days on treated diet, then transferred to clean diet was 14.2 mg ± 10.6 at 10 days.

DISCUSSION

The necessity for the elimination of all viral contamination was demonstrated by the results of the study on disease spread in which a small number of infected larvae caused a high incidence of disease in adults. The data showed that both the diet and eggs used in the facility were contaminated by CPV. The viral contamination associated with the diet was not pursued because a review of the diet preparation facilities suggested that the virus was an airborne contaminant occurring on the surface of the diet.

The results of the studies on egg treatment indicate that the virus was transferred on the surface of the egg and/or on portions of floating eggs, scales, larvae, and other debris that did not directly contact the sodium hypochlorite solution. It should be pointed out that most of the "floating" material was believed to be suspended by the surface tension of the water. The addition of Tween 80® was sufficient to submerge the majority of eggs and scales. It was demonstrated that the contamination could be eliminated from eggs using the 1.0 percent sodium hypochlorite and surfactant, and decanting away all floating matter.

Below is an outline of an egg treatment procedure that we have shown to greatly reduce or eliminate contamination by CPV that is associated with the surface sterilization of large numbers of pink bollworm eggs.

1. Egg pads should be rinsed in running water to remove loose scales, eggs, and fecal material.
2. Egg pads should be submerged for 10 minutes in 1.0 percent sodium hypochlorite treatment solution containing 0.025 percent Tween 80® maintained at 29±1°C. Eggs should be separated from the pads by agitation of the pads for a 3-8 minute period following the initiation of the treatment.
3. After the eggs have settled, all floating material should be decanted away and discarded.
4. Rinse eggs with 0.025 percent Tween 80® to keep eggs submerged and remove the hypochlorite solution.

These procedures were incorporated into the APHIS egg handling procedures December 20, 1974. Table 5 shows the production yields at APHIS prior to and approximately 1 month following the adoption of the modified procedure. A substantial increase in the percent yield occurred in all maintenance units after the new treatment was initiated. In addition, the high yields of 27-39 percent that occurred on certain days suggests that high yields are obtainable when more optimal conditions occur. It should be pointed out that during the period of improved yields, the only change in the diet preparation room was the application and observance of more stringent sanitation procedures.

Table 5.—Summary of the egg-to-pupae yields from three production units of the APHIS Pink Bollworm Rearing Facility 6 months previous and after the modified NaClO egg treatment was initiated[1]

NaClO treatment[2]			Modified NaClO treatment[3]			Percent increase in yield
Production unit	Estimated mean percent yield	Range	No. days used	Estimated mean percent yield	Range	
A	17.1	4.0-32.3	26	23.6	9.1-33.8	40
B	15.4	1.4-32.0	33	21.6	4.1-32.8	40
C	11.6	0.9-27.3	30	24.4	5.4-39.1	110

[1] All yields are volumetric estimates based on 18,000 eggs/ml and 37 pupae/ml.
[2] Data from June 1 to November 29, 1974.
[3] Treatment began December 20, 1974.

LITERATURE CITED

(1) Adams, J. R., Wilson, T. A., Bullock, H. R., and Dulmage, H. T.
1969. transovum transmission of a polyhedrosis virus and microspordian parasite in the pink bollworm and corn earworm. Proc. of 27th Ann. EMSA, pp. 380-381.

(2) Aruga, H., and Nagashima, E.
1962. generation-to-generation transmission of the cytoplasmic polyhedrosis virus of *Bombyx mori* (Linnaeus). J. Insect Pathol. 4: 313-320.

(3) Bell, M. R.
1975. unpublished Ph.D. Dissertation Research. Department of Entomology, Mississippi State University.

(4) Bullock, H. R., Mangum, C. L., and Guerra, A. A.
1969. treatment of eggs of the pink bollworm, *Pectinophora gossypiella*, with formaldehyde to prevent infection with a cytoplasmic polyhedrosis virus. J. Invertebr. Pathol. 14: 271-273.

(5) Martinez, E. and Steurmer, C. W., Jr.
1970. cytoplasmic-polyhedrosis virus and the development and fecundity of the pink bollworm. J. Invertebr. Pathol. 15: 109-112.

(6) David, W. A. L., Ellaby, S., and Taylor, G.
1972. the fumigant action of formaldehyde incorporated in a semi-synthetic diet on the granulosis virus of *Pieris brassicae* and its evaporation from the diet. J. Invertebr. Pathol. 19: 76-82.

(7) Graham, H. M., and Mangum, C. L.
1971. larval diets containing dyes for tagging pink bollworm moths internally. J. Econ. Entomol. 64: 376-379.

(8) Hukuhora, T.
1962. generation-to-generation transmission of the cytoplasmic polyhedrosis virus of the silkworm, *Bombyx mori* (Linnaeus). J. Insect Pathol. 4: 132-135.

(9) Ignoffo, C. M.
1966. insect viruses. *In* "Insect Colonization and Mass Production". (C. M. Smith, Ed.), pp. 511-512. Academic Press, New York.

(10) _____ and Adams, J. R.
1966. a cytoplasmic-polyhedrosis virus, *Smithiavirus pectinophorae* sp. n. of the pink bollworm, *Pectinophora gossypiella* (Saunders). J. Invertebr. Pathol. 8: 59-66.

(11) Martignoni, M. E., and Milstead, J. E.
1960. quaternary ammonium compounds for the surface sterilization of insects. J. Insect Pathol. 2: 124-133.

(12) Mery, C., and Dulmage, H. T.
1975. transmission, diagnosis, and control of cytoplasmic polyhedrosis virus in colonies of *Heliothis virescens*. J. Invertebr. Pathol. (in press).

(13) Ouye, M. T.
1962. effects of antimicrobial agents on micro-organisms and pink bollworm development. J. Econ. Ent. 55(6): 834-857.

(14) Sikorowski, P. P. O., Andrews, G. L., and Broome, J. R.
1973. transovum transmission of a cytoplasmic polyhedrosis of *Heliothis virescens* (Lepidoptera: Noctuidae). J. Invertebr. Pathol. 21: 41-45.

(15) Vail, P. V., Henneberry, T. J., Kishaba, A. N., and Arakawa, K. Y.
1968. sodium hypochlorite and formalin as antiviral agents against nuclear polyhedrosis virus in larvae of the cabbage looper. J. Invertebr. Pathol. 10: 84-93.

UNITED STATES DEPARTMENT OF AGRICULTURE
Animal and Plant Health Inspection Service
Hyattsville, Maryland 20782

Official Business
Penalty for Private Use, $300

POSTAGE AND FEES PAID
U.S. DEPARTMENT OF
AGRICULTURE
AGR 101